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Fisheries Research Bulletin No. 26

Allelic Variation and Fisheries Management

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By

R. W. Gauldie

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New Zealand Ministry of Agriculture and Fisheries

Allelic Variation and Fisheries Management

As kingfishers catch fire, dragonflies draw flame;
As tumbled over rim in roundy wells
Stones ring; like each tucked string tells, each hung bell's
Bow swung finds tongue to fling out broad its name;
Each mortal thing does one thing and the same:
Deals out that being indoors each one dwells;
Selves—goes itself; myself it speaks and spells,
Crying What I do is me: for that I came.

Gerard Manley Hopkins

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Ministry of Agriculture and Fisheries,
Wellington, New Zealand

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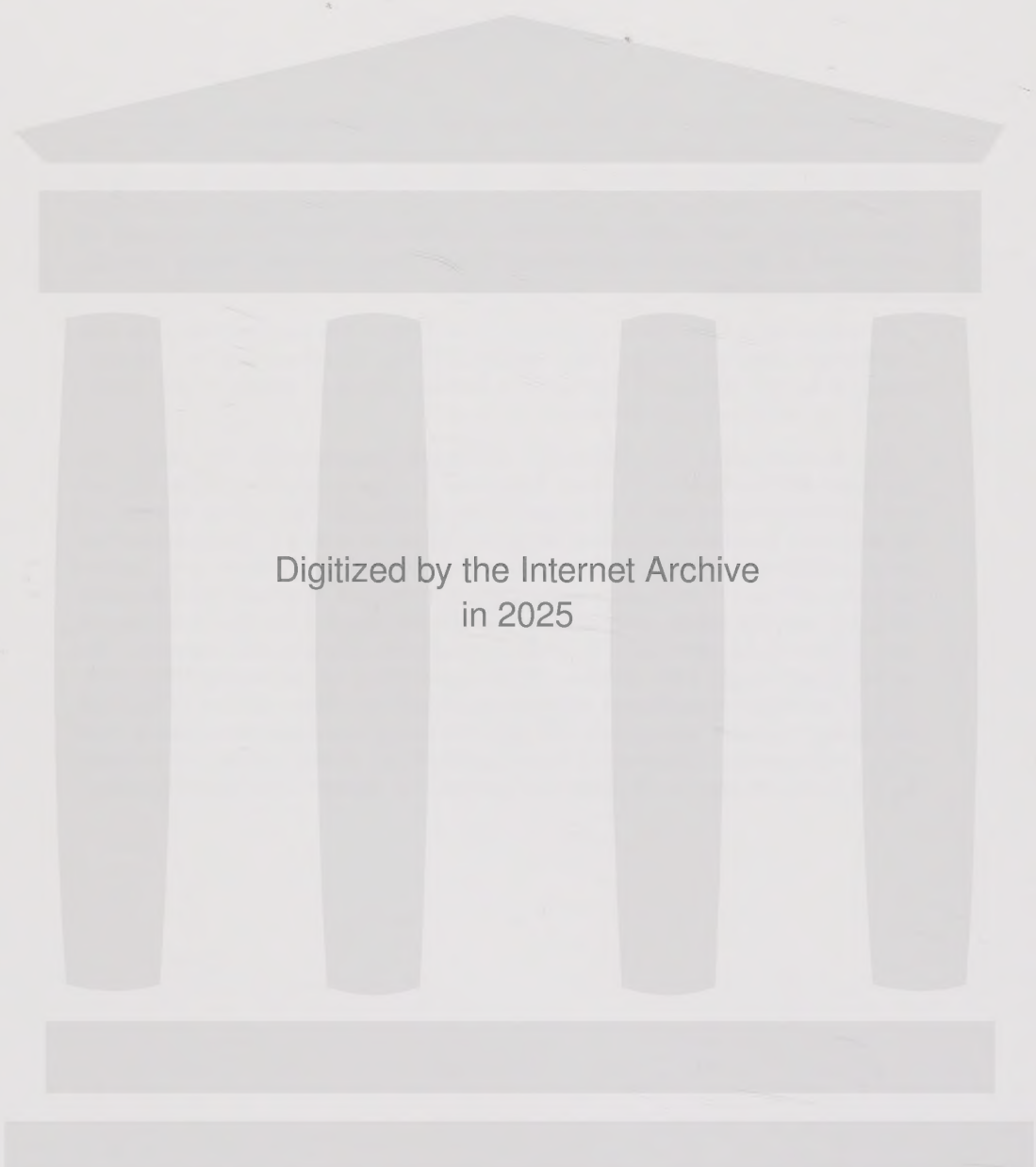
PREFACE

The material in this Bulletin was originally submitted to the *Transactions of the American Fisheries Society* as three separate papers in 1981. The approach to fisheries-oriented genetic studies embodied in those three papers was, and is, a radical departure from the neutral-theory-dominated practices of geneticists working in fisheries problems. The initial response of reviewers to the original papers was largely negative, with one or two significant exceptions.

In response to the direct criticism of reviewers the original material was condensed and its strong tone modified. This condensation was greatly assisted by the editorial efforts of Dr Robert Kendall, editor of the *Transactions of the American Fisheries Society.*

In its condensed form, almost 2 years after submission, the paper was accepted for publication by the American Fisheries Society. However, yet another review resulted in the paper's being rejected. Part of the reason put forward for rejection was that the proceedings of a stock symposium had been published in the meantime (*Canadian Journal of Fisheries and Aquatic Sciences 38 (12)*). There are substantial differences in approach between this Bulletin and the stock symposium. The former deals with the fundamental arguments of the applicability of allele studies to fisheries management; the latter largely deals with reviews of the application of various genetic techniques to fisheries problems without argument for their validity. This, and the strong support for publication that the paper received from senior fisheries management scientists in many parts of the world, led to the decision by Fisheries Research Division to publish the paper in its Bulletin series.



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ABSTRACT

The physiology of alleles discounts their use as genetic tags for stock separation, but points to a better way to understand changes in abundance. A large part of the literature dealing with both theoretical and experimental aspects of allelic variation is examined for its usefulness to fisheries management. It is argued that the use of allelic variation in establishing genetically isolated stocks of fish is inappropriate. Instead, allelic variation represents part of the physiological machinery of the adaptive response of fishes to a complex and changing environment—an environment that includes the fishing industry as a source of mortality. Two case studies are included. One deals with genetic variation at the transferrin locus and the implications of differences in disease resistance among transferrin genotypes. The other deals with genetic variation at the phosphoglucomutase locus and provides evidence for differences in growth rate among phosphoglucomutase genotypes. Genotypic variation in growth rate implies heterogeneity of growth rate within fish populations and seriously compromises the use of average population growth rates drawn from the von Bertalanffy curve or other measures of population growth rate.

INTRODUCTION

Protein alleles* differ in amino acid composition, which allows them to be distinguished by electrophoresis. The use of protein alleles in fisheries management depends on whether or not the alleles are "neutral"; that is, whether or not their biochemical functions—and the physiology of the organism bearing them—are unaffected by changes in their amino acid complement.

If alleles are neutral markers of the genome they can be used to identify separate breeding populations, or "stocks", within a species. The existence of stocks is tested with samples taken over a species' range. The sampling process can follow several statistical models (Gorman and Renzi 1979, Allendorf and Utter 1979) with different sample sizes, but, basically, all these models measure the overall variation in allele frequency at one or many loci in terms of some statistical interrelationship among frequencies. Statistics ranging from simple chi-squared values to multivariate analysis can be used to test whether or not the differences in allele frequencies among geographical areas are greater than expected by chance. Examples of stocks defined in this way are given by Jamieson and Turner (1978), Allendorf and Utter (1979), and Grant and Utter (1980).

If, on the other hand, alleles are not neutral—if they have different biochemistries and impart different physiologies—their frequencies cannot be trusted to reveal true panmictic stocks. When alleles differentially affect, say, growth or fecundity or resistance to disease, their frequencies can vary over space and time **within** a panmictic stock as a result of local selection. It still could be possible to find statistically distinct units within genetically heterogeneous populations, but such units would not have the same meaning for fisheries management as a stock defined by neutral alleles.

Thus, two quite different fisheries management problems are accommodated if allele variation is regarded as either neutral or subject to selection. "Neutralists" and "selectionists" hold these respective viewpoints, and there is controversy between them (Lewontin 1974). Most published studies of allele variation of fishes have, implicitly or explicitly, a neutralist basis, because allele variants are treated as simple population characters whose frequencies can be used statistically to assign stocks within a species. This is not to say that most authors claim that allelic variation is always neutral, although many do. Nonetheless, the final outcomes of nearly all such studies are statements about the statistical significance of differences in mean allele frequencies among samples, which implies that allele frequencies are constant over time.

My purpose here is to caution against ready acceptance of allele neutrality in fisheries management. I believe the evidence is strong, and growing, that much allele variation is accompanied by biochemical and physiological differences, some of which have adaptive importance to organisms. Further, I feel that the theoretical and statical underpinnings of the neutrality approach are greater than is generally acknowledged. With the review and examples that follow, I hope to provoke a reassessment of commonly held assumptions about variation among alleles.

*Alleles are defined, in this context, as alternative proteins coded by different deoxynucleic acid sequences that comprise synonymous genes occurring at the same locus. This is the common usage in the fish literature (Allendorf and Utter 1979), although "allele" properly refers to the gene coding for the protein.

PHYSIOLOGICAL DIFFERENCES AMONG ALLELES

Lewontin (1974), discussing the genetic basis of evolutionary change, sought to establish whether or not models based on neutrality could account for the observed variation in allele frequencies as well as those models based on selection. He concluded that they could.

This is not the question I am addressing. I regard Lewontin's study not as a demonstration that selection is irrelevant in this context, but that particular models based on the classic Haldane-Fisher-Wright* definition of selection do not explain the distributions of alleles any better than the null hypothesis provided by the neutrality theory. In any case, a model of selection that is consistent with the data is a necessary prerequisite neither to the question of whether there are physiological differences among alleles, nor to the application of such differences to fisheries management problems. It is the reverse that is necessary. Realistic theoretical models cannot be made if there is no understanding of the empirical basis for physiological differences among alleles.

The most clearly demonstrated case of physiological variation among individuals with different enzyme alleles concerns leucine amino peptidase in the mussel *Mytilus edulis* and the geographical distribution of alleles at that locus (Koehn 1978, Koehn *et al.* 1980, Koehn and Immerman 1981, Koehn and Siebenaller 1981).

Koehn showed that different leucine amino peptidase genotypes have different physiologies and that these different physiologies are associated with the rapid establishment of regulatory responses to changes in nitrogen metabolism in different environments.

For the rainbow trout, *Salmo gairdneri*, numerous studies have shown biochemical, behavioural, and physiological differences between individuals carrying different lactate dehydrogenase alleles (Northcote *et al.* 1970, Tsuyuki and Williscroft 1973, Huzyk and Tsuyuki 1974, Bailey *et al.* 1976, Tsuyuki and Williscroft 1977, Kao and Farley 1978, Klar *et al.*1979a, 1979b, Redding and Schreck 1979, Northcote 1981, Northcote and Kelso 1981). Rainbow trout show differences in swimming stamina associated with lactate dehydrogenase genotype that is thought to affect habitat choice both in terms of fast stream-slow stream options and upstream and downstream migration in the early life history of the fish.

Similar studies in other fishes show a correspondence between the dependence of allele function on environmental factors like temperature, and the geographical distribution of those alleles. These fishes include the mummichog, *Fundulus heteroclitus*, (Mitton and Koehn 1975, Powers and Powers 1975, Place and Powers 1978, Powers and Place 1978, Powers *et al.* 1979), the high cockscomb, *Anoplarchus purpurescens*, (Johnson 1971, 1977), the sand shiner, *Notropis stramineus*, (Koehn 1970, Koehn *et al.* 1971), the red shiner, *Notropis lutrensis*, (Richmond and Zimmerman 1978), and the fathead minnow, *Pimephales promelas*, (Merritt 1972). Most of these studies have been of lactate dehydrogenase variants. In a way, lactate dehydrogenase has been a victim of its own success. Once it was known that lactate dehydrogenase variants were under selection, more studies on them were initiated. There is a danger in this approach that lactate dehydrogenase will become regarded as a special case, since little experimental effort is expended on other fish enzyme variants and consequently there are no published data. There have already been suggestions (Tsuyuki pers. comm.) that lactate dehydrogenase is an "end-point" enzyme and hence part of a special class of enzymes that can vary genetically while others are constrained. However, in animals other than fish, allelic variants at other loci have been shown to be directly acted on by selection (Leigh Brown 1977 (phosphoglucomutase), Alahiotis *et al.* 1977 (α-glycerophosphate dehydrogenase), Morgan 1975 (alcohol dehydrogenase)).

For the protein transferrin, recent studies have shown that different alleles confer different resistance to infection by various pathogens (Suzomoto *et al.* 1977, Pratschner 1978). Transferrins have been known as bacteriocidal agents for a long time and allelic variation of transferrin in humans, birds, and farm animals is widely accepted as being due to differences in disease resistance and differences in some "general condition" factor in animal physiology. This topic, and its consequences for fisheries stock studies where transferrins are widely used, will be treated more fully later in this bulletin.

*Haldane, Fisher, and Wright did not have convergent views on selection, but they did use the same formal definition of selection, although with different notation (Wright 1969, pp. 28–31).

Many studies show clear geographical clines in allele frequencies or heterozygosities. Clines need not be inconsistent with the neutrality theory, but in many studies a particular environmental variable is correlated with allele frequencies, which suggests the variation is maintained by selection (Fujino and Kang 1968, Lush 1969, Pantelouris *et al.* 1971, Avise and Selander 1972, Koehn and Mitton 1972, Frydenberg *et al.* 1973, Koehn *et al.* 1973, Christiansen and Frydenberg 1974, Hjorth and Simonsen 1975, Mathers 1975, Murphy 1976, Yardley and Hubbs 1976, Schopf 1977, Wilkins 1977, Buth and Burr 1978, Koehn and Williams 1978, Lassen and Turano 1978, Powers and Place 1978, Shami and Beardmore 1978, Shoubridge and Leggett 1978, Avise and Felley 1979, Hadfield *et al.* 1979, Kijima and Fujio 1979, Krueger and Menzel 1979, Gosling 1980, Gauldie and Johnston 1980, South Pacific Commission 1981).

The behaviour of allele frequencies in terms of the heterozygosity being controlled by the variation in the environment rather than a formal model of balanced polymorphism based on an excess of heterozygotes has been examined by a number of authors (Levins and MacArthur 1966, Gillespie and Langley 1974, Soulé 1976, Valentine 1976, Minawa and Birley 1978, Powell and Wistrand 1978, Pierce and Mitton 1979, Mackay 1980). Some studies have shown that there is year-to-year variation in allele frequency in some fishes which one might expect from heterozygosities that are a function of environmental variance (Wilkins and Iles 1966, Elson 1973, Loch 1974, Kristiansson and McIntyre 1976, Wilkins 1977, Jamieson and Turner 1978, Siebenaller 1978, Smith 1979, Yoshiyasu 1979, Gauldie and Johnston 1980, Grant *et al.* 1980).

There are many reports of differences in allele frequency between, and within, populations in which there is no direct test of, or even argument about, whether the observed variation in allele frequency is due to neutral or selective effects. Instead, these reports rely on the Hardy-Weinberg equilibrium test, or the assumption that the level of heterozygosity (as a measure of genetic variation) is dependent on the "complexity" of the environment, or simply assume that the variation has no physiological significance. To restrict the size of this review, I will not list all of these reports, but simply characterise them as I have done above.

A few reports describe populations that have stable allele frequencies either over distance or time (Smith and Jamieson 1978, Grant and Utter 1980, Sidell *et al.* 1980) or are virtually fixed at many loci (Smith and McKoy 1978, Felley and Avise 1980). There may be many more invariant species that have not been reported, since no variation is often regarded as no result.

Thus, much experimental evidence supports an interpretation of genetic variation of fish and molluscs in terms of physiological difference among alleles. Similar arguments hold for allele variation in some other organisms (Kojima and Tobari 1969, Leigh Brown 1977, Bijlsma 1978, Kamping and van Delden 1978, Bijlsma and van der Muelen-Bruijns 1979, Danford and Beardmore 1979, Fucci *et al.* 1979, Lee and Pegoraro 1979, Mason 1979, Narise 1979, Barker and East 1980, Hoorn and Scharloo 1980).

NEUTRALITY MODELS

Not all neutrality models of biochemical function are theoretical. Three important empirical neutral arguments concern the dependence of allele variation on molecular weight, the molecular clock analogy, and the segregational load. These empirical arguments have had a great influence on the actual analysis of allele-frequency data in fisheries management and so require further comment.

ALLELE VARIATION AND MOLECULAR WEIGHT

It is reasonable to suppose that if allelic variants of a protein are just chance changes in amino acids without any biochemical significance, the number of alleles should increase with increasing molecular weight of the protein. This hypothesis is easy to test because molecular weights of a given enzyme typically do not vary greatly between widely different animals. For example, the molecular weights of various lactate dehydrogenase enzymes vary by only a few percent between salmon and humans (Boyer 1975). However, the variation in the number of alleles found for a particular protein (or proteins of similar size) (Ward 1978) is very great indeed. Gauldie (in press) surveyed frequencies of glucosephosphate isomerase alleles in populations of 81 species; the number of alleles per species ranged from 1 to 7 and heterozygosities ranged from 0.0 to 0.81 among species. The number of alleles per locus shown by glucosephosphate isomerase is by no means unusually large. The transferrin locus has 17 alleles in the Atlantic cod, *Gadus morhua*, (Jamieson and Turner 1978). Sheep and cattle have almost as many transferrin alleles, but other vertebrates, such as birds (Frelinger 1973) and Atlantic salmon, *Salmo salar* (Payne *et al.* 1971), have only two.

In the face of this kind of within-locus variation, one would expect to find low correlations between the number of alleles and the molecular weight of proteins. It is not surprising, then, that the r^2 values for correlations between molecular weight and heterozygosity are low, 0.003 to 0.155 (Nei *et al.* 1978). The average correlations for species listed by Nei *et al.* are 0.079–0.522, but the highest value, 0.522 for *Drosophila* sp., is claimed by Leigh Brown and Langley (1979) to be an overestimate for which the true value should be 0.11. Leigh Brown and Langley also point out that such a low correlation is just as readily predicted by other, including selectionist, hypotheses.

Nei *et al.* tried to explain these observations, including that by Hopkinson *et al.* (1976) that there was no significant difference in molecular weight between polymorphic and monomorphic loci in humans, by arguing that a strong correlation is masked by the stochastic loss of alleles (drift effects) due to differences in effective population size. The argument is somewhat roundabout. Effective population size cannot be measured experimentally; it has to be estimated from the distribution of allele frequencies. Because heterozygosity is an indirect measure of allele frequency, it must be highly correlated with estimated effective population size. An estimate of the stochastic loss measured via heterozygosity from the effective population size cannot be applied to the original data set without invoking a circular argument. Nei *et al.* avoid this circularity only by introducing other circularities. They obtain the theoretical value of the proportion of allelic variation that is explained by molecular weight, "if we assume that the mutation rate for a locus is proportional to the molecular weight of the polypeptide produced", which is, of course, the very thing they set out to test.

I conclude that the neutralist explanation that low correlations between allele variation and molecular weight are due to drift is weak.

In contrast to the properties assigned to it in neutrality arguments, molecular weight is correlated with certain physiological properties of proteins, particularly stability in solution and net alkaline or acid surface charge (Dice *et al.* 1973, Momany *et al.* 1976, Killilea *et al.* 1979, Rocha *et al.* 1979). Consequently, one might look for some correlation between allelic variation and physiological function, and one can find it in the allelic variation reported by Sharp and Pirages (1978) for some of the species in the family Scombridae.

Sharp and Pirages list the number of electrophoretic variants at 21 loci (14 enzymes) for 18 species, which include both the highly specialised homeotherms like *Thunnus thynnus* and unspecialised poikilotherms like the sierras and bar mackerels (*Scomberomorus* spp.) (Table 1). The mobilities of all electrophoretic variants were measured relative to the corresponding locus of the yellowfin tuna, *Thunnus albacares*.

TABLE 1: Average number of alleles per locus (21 surveyed) for species in the family Scombridae (from Sharp and Pirages 1978)

Av. No. of alleles per locus	Species
1.29	*Thunnus (Neothunnus)* thynnus* (1)†; *Thunnus (Neothunnus)* maccoyi* (1)†
1.35	*Thunnus (Neothunnus)* orientalis*
1.37	*Euthynnus affinis*
1.39	*Thunnus (Thunnus)* tonggol* (1)†
1.41	*Scomberomorus concolor*
1.43	*Thunnus (Neothunnus)* alalunga* (2)†; *Thunnus (Thunnus)* albacares* (2)†
1.45	*Acanthocybium solandri*
1.47	*Sarda chiliensis*; *Thunnus obesus*
1.51	*Thunnus (Thunnus)* atlanticus* (1)† or (2)†
1.53	*Scomberomorus cavalla*
1.55	*Scomber japonicus*
1.71	*Katsuwonus pelamis* (*Euthynnus pelamis* in Robins *et al.* 1980)
1.75	*Auxis thazard*; *Euthynnus lineatus*
1.81	*Scomberomorus sierra*

*Subgenera from Collette (1978).
†Heat exchange capabilities: (1) indicates relatively advanced; (2) indicates relatively primitive (after Collette 1978).

To test the relationship between number of alleles and molecular weight for Sharp and Pirages' scombrids, I used enzyme molecular weights for humans (Harris and Hopkinson 1976), because few such data exist explicitly for scombrids. Sequencing data from Boyer (1975) indicate that the molecular weights of lactate dehydrogenase and malate dehydrogenase in beef differ from those in tuna and salmon by less than 5%. A positive association between the number of alleles and molecular weight is not supported (Fig. 1).

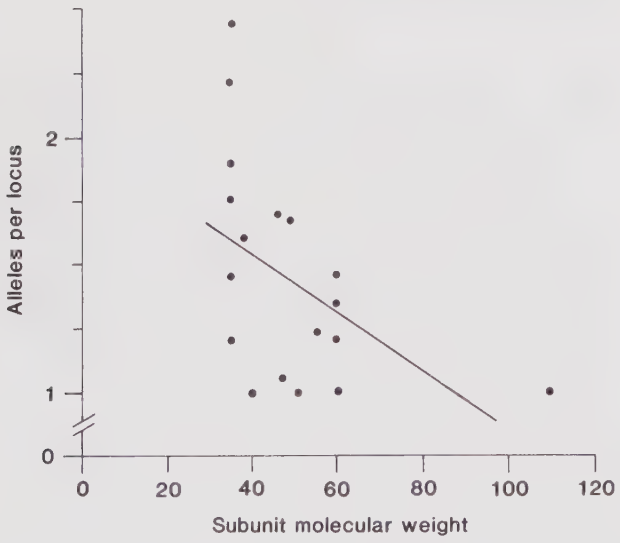

Fig. 1: Average number of alleles per locus reported for scombrids (Sharp and Pirages 1978) plotted against subunit molecular weight. The slope of the regression line is significantly greater than zero ($P < 0.001$).

The family Scombridae includes unspecialised poikilotherms like *Scomber japonicus* and species like *Thunnus thynnus* that are relatively homeothermic. If the number of alleles per locus is a function of the environment, one would expect a progressive decline in the number of alleles per locus from the poikilothermic to the more homeothermic scombrids. In a general way, Table 1 does suggest this trend, but with many exceptions. The trend is more consistent for closely related species. Among Sharp and Pirages' data, two subgenera, *Thunnus* and *Neothunnus*, provide enough species (three and four respectively) to test. Within subgenus *Neothunnus*, the bluefin species *Thunnus thynnus* and *Thunnus maccoyi* are less variable than their cogener *Thunnus alalunga*, and there is evidence (Collette 1978) that the bluefins have the more advanced heat exchange system. Similarly, in subgenus *Thunnus*, the more advanced *Thunnus tonggol* has fewer variants than its less sophisticated homeothermic cogener *Thunnus albacares* (Table 1).

These speculations about scombrids are seriously compromised by the uneven sample sizes of Sharp and Pirages' study, but trends that emerge cast considerable doubt on any simple relationship between allelic variation and molecular weight. On the contrary, trends are consistent with the view that polymorphism is tied to the environment and physiology of scombrids.

In general, empirical relationships between allele variation and the molecular weight of proteins cannot be regarded as favourable evidence for the neutrality theory.

THE "MOLECULAR CLOCK" ANALOGY

Genes, and the biochemicals they mediate, change with time; the longer species have been separated from one another, the greater will be the molecular differences between them. This is the idea of the molecular clock. It is buttressed by correlations of "genetic distances" (in the literal rather than the formal sense of Rogers 1972) with taxonomic differences between species, genetic distance being measured on parts of the genome that are independent of the phenotypic characters used in taxonomy. The best known of these correlations was derived by Fitch and Margoliash (1967), who showed that divergences between animals measured by accumulations of amino acid substitutions paralleled their phylogenetic divergences measured by phenotype taxonomy.

The molecular clock analogy has been taken as support for the neutrality of allele variation (for example, by Kimura and Ohta 1971) because of its

implication that genetic differences are simple functions of time, without short-term meaning for the physiology of organisms. There are at least two problems with this view, however. First, biochemical changes, such as amino acid substitutions, can change the physiology of an organism, as I discussed previously. Second, although the molecular clock idea may have provoked useful correlations between genetic distance and phenotype taxonomy, the correlations cannot be used backwards to invoke either molecular clocks or allele neutrality. This is because none of the measurements entering the correlations have dimensions of time (and so cannot relate the magnitude of differences to the time since the differences arose), and because the same correlations are conceivable if selection operated on any or all levels of biological form and function.

Thus, the molecular clock analogy is ambiguous—perhaps irrelevant—with respect to arguments between neutralists and selectionists.

SEGREGATIONAL LOAD

The concept of segregational load is the very basis of the neutrality theory. It first appears in Kimura (1960) as a development of the genetic-load concepts of Muller (1950) and Haldane (1957). The argument is simple: if all the genes in an organism were polymorphic, and if that polymorphism were maintained by selection, each randomly mating population would produce a vast number of different combinations of genotypes from all the segregating loci.

For selection to restore the parental frequency distribution from the multitude of offspring patterns, an enormous mortality is required. An indication of the size of these mortalities is illustrated by an example from Kimura and Ohta (1971): "To carry out mutant substitution [effectively, a new allele] at the above rate, each parent must leave $e^{180} = 10^{78}$ offspring for only one of the offspring to survive".

In humans the average heterozygosity per locus is about 8% for 91 loci (Harris and Hopkinson 1976). The neutrality argument is that the mortality necessary to maintain that much variation at every locus is greater than is ever possible for humans; therefore, the observed heterozygosity must be due to some non-selective process (Kimura and Ohta 1971).

In his earlier attempts to deal with the same problem, Fisher (1958) arrived at a similar conclusion. He recognised a paradox (one might characterise this whole problem as Mendel's paradox) arising from the random mating process. On the one hand a large number of new genotypes arise from Mendelian segregation; on the other hand the phenotype of the randomly mating population maintains the same distribution. Is this uniform phenotype maintained by the selective removal of those genotypes that would change the phenotype distribution? Fisher's answer is no, and yes. He regarded allelic variants as being almost physiologically identical. If the uniform phenotype arises from all the genotypes being virtually physiologically identical, how does selection work? Fisher (1958) answers with a curious argument based on an analogy with the "psycho-physical" perception of differences in the weight of objects. This argument concludes: "The selective advantage will increase or decrease continuously, even for changes much smaller than those appreciable to our own senses, or to those of the predator or other animal, which may enter into the biological situation concerned."

At first sight this statement does not make any logical sense. How can selection operate on such invisible differences?

The key to understanding both Kimura's and Fisher's explanations of allelic variation (Fisher refers to "allelomorphs") lies in the kind of biochemical machinery that they were modelling.

Unfortunately, neither Kimura (1960) nor Fisher tells us in plain words what sort of biochemistry he had in mind, but their biochemistry can be inferred from their models. It is clear that they envisioned enzyme functions as operating independently of each other and having additive net effects. A change in one direction by one enzyme allele could be offset in a simple additive fashion by another enzyme allele at another locus. No known biochemistry functions like that. Atkinson (1976, 1977) clearly shows that biochemical pathways are highly integrated and can be regarded as goal oriented. The biochemistry of organisms is not a soup whose flavour can be averaged out over its ingredients.

Kimura and Ohta's (1971) argument based on the figure of 8% average heterozygosity per human locus is misleading, since it is made in the context of biochemistry that is, as far as we know, non-existent. Of the 92 loci reported by Harris and Hopkinson (1976), 61 are monomorphic, so that most of the heterozygosity is carried by a few loci which, with rare exceptions, have only 2 alleles (Fig. 2).

The argument for the existence of endless possible combinations of alleles, because of some variation at every locus, is therefore also misleading, since it refers to an unacceptable concept of biochemistry. The use of heterozygosity as an overall measure of allelic variation is in part to blame. The

categorisation of the data in this manner by Kimura (1960) ensures the consequence of his argument.

It is also misleading to assume that the interactions between all loci are additive. Ironically, the same issue of *Journal of Genetics* that carried the paper by Kimura (1960) also had a paper by Gilbert (1960), who demonstrated experimentally that gene effects are not linear and not additive. Spickett and Thoday (1966) confirmed this non-additivity in a much more extensive experiment. More recently I have been able to demonstrate directly that allelic variation at four loci involved in glucose-6-phosphate metabolism is not independent and that the gene-to-gene interactions are non-linear and non-additive (Gauldie in press).

Theoretical genetic models deal only with gene frequencies, but in practical applications one also must consider gene-product biochemistry and the physiology and behaviour resulting from that biochemistry. Kimura, who largely was responsible for the development of the neutrality theory, has avoided using it as anything other than a theoretical description of gene frequency itself. It is possible that when relationships of gene-product biochemistry to physiology and behaviour are better understood in quantitative terms, the mathematical base of the neutrality theory can be extended out into more complex aspects of population biology.

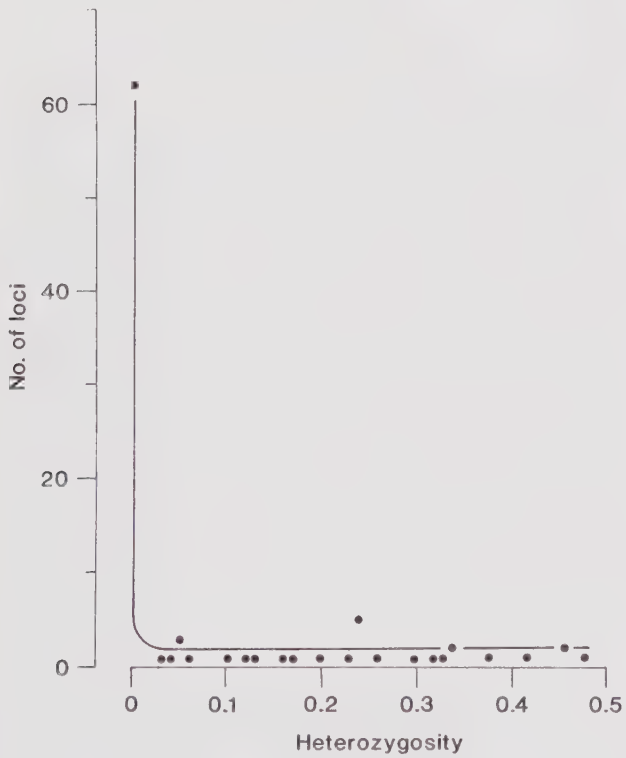

Fig. 2: The number of loci with different levels of heterozygosity found in humans (derived from Harris and Hopkinson 1976).

Unlike Kimura, some fisheries geneticists (for example, Allendorf and Utter 1979) have applied the neutrality model too literally. When simple, additive biochemistry is assumed in fisheries management studies, the use of average heterozygosities has effectively blanked out differences between year classes (or between sequential samples). The regional allele-frequency averages that result are artificial and discount the often enormous variance within the regions that was to be characterised. For example, Grant *et al.* (1980), in a study of the stock identification of sockeye salmon, *Oncorhynchus nerka*, reported a large amount of year-to-year variation at four loci—glutamate pyruvate transaminase, phosphoglucomutase, lactate dehydrogenase, and phosphoglucose isomerase—in fishes from stations within the Kasilof River drainage, Alaska. These four loci show an average difference of 7% in the principal allele frequency between samples taken in 1975 and 1976. The range in average difference is from 0 (in 4 of 20 samples) to almost 44%. The authors dismiss this variation: "We tested this assumption in the Kasilof River by sampling the same locations for two consecutive years and found no significant difference between years." They were able to make this statement because they calculated the heterozygosity over these 4 loci and 22 others that were virtually monomorphic. They assert that "the estimates are based on genetic data which are not subject to direct environmental influences and which appear to be stable over time."

Clearly we require realistic biochemical models of what allelic variants actually do. Even the simplest kind of study, of the interdependence of allele variation at the biochemically juxtaposed loci phosphoglucomutase and glucosephosphate isomerase, reveals surprisingly complicated interactions (Gauldie in press).

STATISTICAL LIMITATIONS

When alleles are considered in fisheries management on the basis of their physiological differences rather than their frequency distributions, several statistical problems can be overcome. For example, an observation could be made that there is a correlation between the allele genotype and growth, or disease resistance, so that an exact measure of allele frequency in a population sample is not so critical. In contrast, use of allele phenotypes as neutral markers of populations requires not only an exact measure of the allele frequency itself, but also an estimate of its reliability.

The problem of statistical reliability of allele frequency has been discussed by Lewontin (1974) and Fairbairn (1979) as a largely theoretical problem. However, just as the issue of physiological differences versus neutrality of allele variations can be approached experimentally, so can the problem of reliability. At least four sources of error exist: year-to-year variation; estimation of electrophoretic patterns from gels; small sample size; and hidden genetic variation.

The problem of year-to-year variation was addressed above and will be considered in more detail with respect to transferrin alleles later. Year-to-year variation is common, and the variance that it introduces can be considerable.

The reliability when electrophoretic patterns are estimated from gels hinges on two points: the criterion whereby a pair of electrophoretic bands are considered to be a thick single band, or two thin single bands; and the proportion of unresolvable individuals within a sample. Gauldie and Johnston (1980) used the "naive person" test and found that several electrophoretic phenotypes could not be resolved, even after more protein was dissolved. (The naive person test entails asking someone who is not familiar with the vagaries of gel patterns to indicate which samples have bands that can be clearly counted. This type of rigour is particularly important for marine fish for which breeding experiments cannot be performed.)

For example, the proportion of "unresolvable" phosphoglucose isomerase patterns for *Thyrsites atun* from nine stations had a Poisson distribution with a mean of 0.036, a standard deviation of 0.19, and standard error of 0.02. If the undetected error in identifying alleles is at least as large as the detected error, the mean should be twice as large, the variance twice as large, and the standard deviation and standard error 0.14 times as large. The consequent range of expected variation that may be due to chance is 0.10, which means that only two samples reported by Gauldie and Johnston (1980) for *Thyrsites atun* are statistically significantly different, the highest and lowest frequencies. If the confidence limits are based on the standard deviation, none of the frequencies observed is significantly different from the mean value. The number of unresolvable rejects in the more extended sampling reported by Gauldie and Johnston for *Cheilodactylus macropterus* is lower, but the maximum frequency range of the three alleles reported is small, 0.09 to 0.23, so that the statistical analysis is seriously compromised by this source of error. In addition, heterozygotes are more likely to be unreadable, and so there is also a potential bias towards losing the less common alleles.

The reliability of a proportion drawn from a sample when the entire population size, or even its likely lower limit, is known can be estimated from the formula $\sigma^2 = [(N - n)/Nn]pq$. The minimum estimate of population size of the spawning aggregate from which the Pegasus Bay sample (JO409) of *Cheilodactylus macropterus* (Gauldie and Johnston 1980) was drawn is 500 000 fish. For a sample size of 500 drawn from this population, we have for the S allele a frequency estimate of 0.14, and a standard deviation of 0.015. The minimum difference between any frequency estimate for samples of this size (which is very large) is thus 0.06. For smaller samples like the Cloudy Bay sample (JO401) of 94, the standard deviation of the S allele frequency for a population of 500 000 spawners is 0.039, with a consequent minimum detectable difference of 0.15 in allele frequency.

These confidence limits can be calculated only on the assumption that the sample is drawn randomly. For a large spawning aggregate of marine fishes, neither spawning nor sampling can be random because the area over which a particular spawning episode takes place is too large. It may be that this kind of heterogeneity is effectively randomised over a number of years, but little evidence has been collected to establish random mating in fishes.

It is evident that treating allele-frequency distributions as a purely statistical problem probably requires a much more rigorous approach than is to be found in the literature dealing with stock sepa-

ration. Indeed, the prior information necessary to treat the problem of allele frequencies statistically is usually the very information that the statistical analyses are intended to provide: the boundaries of the stock.

Exploiting the knowledge of physiological differences between alleles removes some of these statistical problems. More information is provided by examining relationships like that between allelic phenotype and growth rate. Statistical problems still remain, but they now centre around the issue of how much of the variation in one factor can be explained by another.

All the statistics employed in the analysis of allele frequencies assume that a random sample has been taken. This is particularly true of multivariate statistics like genetic distance (Rogers 1972) and studies, like those of Gorman and Renzi (1979), in which statistics based on very small samples ($\geqq$ 10) are used to characterise populations. A random sampling programme is necessary to establish such statistical bounds of a population, but the only way a random sampling programme can be devised is when the population bounds already are known, such as whether a genetically isolated population comprises 50, 500, 5000, 50 000, or 500 000 fish.

A further, and more severe, defect of genetic distance measures lies in the way that genes interact. All of the genetic distance models reviewed by Rogers (1972) are essentially a normalised covariance calculated on the classical linear additive model of gene interaction assumed by Fisher (1958) and Kimura and Ohta (1971). It is clear from this review that the linear additive model of gene interaction is not biologically meaningful, and as a consequence, nor are the genetic distances based on such models.

"Hidden" allelic variation has been reported at a number of loci. These are alleles that run together at the same speed during electrophoresis so that they do not stain as separate bands. Hidden alleles are detected by removal, by denaturation, of electromorphs that migrate at the same speed, or by altered buffer chemistry or pH. Denaturation-type hidden alleles are described by Thatcher (1977), Coyne and Felton (1977), Johnson (1978), and Kreitman (1980). From a neutralist point of view any inherited variation that has no physiological consequence provides information about genetically isolated stocks. But if these hidden alleles represent post-translational modification, some kind of functional differences might be suspected.

IMPLICATIONS OF PHYSIOLOGICAL DIFFERENCES AMONG ALLELES FOR FISHERIES MANAGEMENT

Irrespective of the conclusions of theoretical arguments in genetics, there is experimental evidence for physiological differences between alleles.

I will introduce two case studies that demonstrate the consequences of this evidence to the practical business of fisheries management. The first is an example of the statistical and interpretive problems that arise when only neutralist considerations are applied to a particular locus, transferrin, whose alleles are known to be associated with physiological differences of selective significance; and the advantages of taking a selectionist approach. The second case study demonstrates a statistical association between growth rate and phosphoglucomutase genotype in the tarakihi, *Cheilodactylus macropterus*.

The significance of these case studies lies in showing that allele frequency is not a reliable means of identifying genetically isolated stocks of fish. The necessity of genetically isolated stocks to fisheries management strategy lies in the following extrapolation: if the stock is a self-contained, randomly mating population with respect to one part of the genome, it must be also for other parts of the genome, particularly the parts that relate to growth and mortality. In this way identification of a genetically isolated stock assures the fisheries manager that the population is homogeneous with respect to growth rate; that is, any variation in fitted growth parameter is purely stochastic. If, on the other hand, populations vary in genotype over time and space in one part of the genome, they are likely to do so at other parts of the genome, like the parts associated with growth rate. Under these circumstances the very presence of more than one genotype assures the fisheries manager that the population is genetically heterogeneous. If that genetic heterogeneity can be shown to be associated with growth rate, typical mean population growth rate calculations from the von Bertalanffy curve are likely to be unstable and, in any biological sense, irrelevant. Similarly, mean population mortality and recruitment rates might also be regarded as being highly suspect.

Case Study No. 1: Statistical and interpretational problems arising out of physiological differences between alleles at the transferrin locus

Transferrin polymorphisms were among the first employed in fisheries management studies to define stocks of fish (Møller and Naevdal 1966). Subsequently, transferrins have been used widely for genetic distance modelling (Jamieson and Turner 1978) and for estimating proportions of stocks in the catch (Fairbairn 1979).

Transferrin has two metal binding sites that form complexes with iron (Zapolski *et al.* 1974), zinc (Phillips 1976, Evans 1976), copper (Aasa and Aisen 1968), and chromium, manganese, and cobalt (Aisen *et al.* 1969). There are, in addition to serum transferrins, other transferrins associated with the epithelial cells of the gut mucosal membrane (Finch 1975), in milk, and in avian eggs (Feeney and Allison 1969).

Iron transport by serum transferrins has been most intensively studied. The transferrin molecule has two iron binding sites, each carrying one iron molecule. There are thought to be functional differences between the binding sites such that one site carries iron specifically to the developing erythron in the bone marrow and the other releases iron in transit to polymorphonucleocytes in the blood (Morgan and Baker 1969, Hahn 1973, Awai *et al.* 1975, Lane 1975, Princiotto and Zapolski 1975, Phillips 1976). The release of iron to polymorphonucleocytes is critical to the bacteriocidal role of these cells, perhaps through activation of myeloperoxidases (Chandra 1973). The transport of iron to the biologically appropriate site, and the success of competition for this iron by pathogens, depend on the shape and surface charge of the transferrin molecule (Lane 1972). Although transferrins are large molecules relative to iron (molecule weight ratio 76 000 : 56), their saturation by two iron molecules causes them to expand slightly and to become 25% more spherical (Rosseneu-Motreff *et al.* 1971). Iron withdrawal by the host as a defence against parasites, particularly protozoans and bacteria, is well established (Weinberg 1971, 1975, Ganzoni and Puschmann 1975). Transferrins, with their highly selective iron releasing properties, assume a particularly important role in this defence mechanism by denying iron to invading pathogens (Bullen *et al.* 1978, Calver *et al.* 1978, Leyland *et al.* 1979).

PHYSIOLOGICAL DIFFERENCES

Because conformation and surface charge are critical in the biochemical role of transferrins, one

might expect electrophoretic variants, which differ in charge state, to also differ in their physiologies. This expectation has been confirmed. Most of the evidence comes from birds and mammals.

Frelinger (1972) first showed an *in vitro* differential inhibition of microbial growth by different transferrin alleles isolated from the egg white of the pigeon *Columba livia*. The same effect was described for rat transferrins by Calver *et al.* (1978). Frelinger (1971) also showed that different transferrin genotypes lead to differential survival of squabs due to both their own, and maternally derived, transferrins. Pigeon A and B transferrins differ by a single amino acid (Frelinger 1973). Brown and Sharp (1970) reported an association between transferrin phenotype and glutathione concentrations in the blood of pigeons similar to that described for monkeys (Brown *et al.* 1970).

In cattle, Neethling and Osterhoff (1967) showed that there is a large difference in the iron carrying capacity (that is, affinity for iron under standard conditions) of the three most common cattle alleles in the ratio 1.0 (TfEE) : 2.9 (TfAA) :1.7 (TfFF). Cattle transferrin also shows abnormalities in the electrophoretic mobility of alleles due to changes in the number of surface sialic acid residues (Spooner and Baxter 1969, Stratil and Spooner 1971). Further examination of this abnormality revealed a sialic acid "attaching" enzyme that is itself polymorphic (Spooner *et al.* 1977). Electrophoretic variation in mouse transferrins following infection by *Rickettsia mooseri* may be due to the attachment of sialic acid residues to transferrin (Awaad *et al.* 1978). Sialylation is also involved in electrophoretic variation of transferrins in sheep (Stratil 1973).

Arguments have been made both for and against transferrin genotypes being related to fertility and production in cattle. Ashton (1959) suggested that transferrin genotype of the mother might affect chances of survival of a bovine embryo, and that mature groups of different genotypes might have varying levels of fertility. In a later report (Ashton 1961) he confirmed that parental transferrin genotype did affect fertility, although the mechanism was not clear. Ashton (1961) observed disturbed segregation ratios resulting from different maturing groups and concluded from these that matings between homozygous parents were more fertile than matings between heterozygous parents. He concluded from this that homozygous embryos were more viable than heterozygous embryos. All of Ashton's experiments were conducted in dairy cattle. A similar investigation by Fowle *et al.* (1967) on beef cattle arrived at a different conclusion: transferrin genotype did not affect fertility. However, Ashton's experimental group were artificially bred and had a rather low fertility rate, 54%, compared with the 90% fertility rate of the pasture-bred cows used by Fowle *et al.* (1967). Fowle *et al.* did, however, report a significant difference in milk production between transferrin genotypes similar to that reported by Datta *et al.* (1965) and Kushner *et al.* (1973).

Ashton (1965) makes a strong argument for physiological effects related to transferrin genotype in cattle that include: heterozygote advantage *in utero*, maternal-foetal incompatibility, poorer fertility of heterozygous bulls and cows. Some other reports confirm this general relationship between fertility and transferrin in cattle (for example, Meyer 1967). But there are other publications apart from Fowle *et al.* (1967) that do not support these findings (Buschmann 1965, Boyd *et al.* 1969, Kushner *et al.* 1973). The transferrin-fertility relationship may be breed specific or evident only under physiologically stressful conditions.

The differences in results between Fowle *et al.* (1967) and Ashton (1961) draw attention to a basic experimental problem in population genetics. Experiments conducted on individuals with different genotypes that are maintained under the same conditions try to measure physiological responses of different genotypes to the same environmental conditions. A more appropriate experiment would be to see if different genotypes could produce the same physiological response to different environmental conditions. The morphological similarity of individuals within a species over what is often a wide range of environments strongly implies that individuals are adapted to produce the same response (the species *gestalt*, if you like) under different conditions by use of genotypes with different biochemical, and hence physiological, properties.

Ashton (1959, 1965) has suggested that transferrin genotype influences the adaptability of individuals to different climatic conditions. But a better test of this theory is provided by an experiment by Khattab *et al.* (1964) on the Welsh Mountain sheep flock maintained by the University College of North Wales. These sheep can be described as a representative sample of the "hardy" section of the breed and can be distinguished from the more domesticated ("pedigree unproved") part of the strain by having "more hair and kemp in the fleece and by other less easily definable characters". Khattab *et al.* report a disturbed segregation ratio involving the TfC allele. This disturbance is consistently present in the particular mating where the dam is homozygous for the TfC allele and the sire is heterozygous for the same allele. The Tf locus shows no heterozygote advantage, but in spite of the segregation disturbance, genotype frequencies remain

remarkably constant throughout the range of ages covered by the experiment. Khattab *et al.* refrain from calling this effect selection against the Tf locus, since there is no obvious difference in fitness, yet it is clear that selection takes place. They note: "this lack of any obvious difference in fitness is characteristic of several biochemical polymorphisms so far studied in farm animals. If there are differences in fitness then perhaps they manifest themselves only under severe physiological stress, such as an exceptionally severe winter provides".

Sheep generally appear to have differences in the viability of lambs that are associated with different transferrin genotypes (Rasmusen 1976, Rasmusen and Tucker 1973). The possible physiological effects of those differences include: differential implantation of the embryo; different rates of foetus survival; different rates of survival to sexual maturity; and differences in gamete production and survival (Rasmusen 1976).

In pigs, there is evidence for the transferrin locus being associated with an early lethal factor in farrowing (Christensen 1970, Imlah 1970).

Transferrin polymorphisms have been described in horses (McGuire and Weitkamp 1980), monkeys (Goodman and Wolf 1963, Goodman *et al.* 1965, Brown *et al.* 1970), Australian hopping mice (Baverstock *et al.* 1977), and fur seals (Bogdanov *et al.* 1977), but without reference to fitness differences.

There is evidence of differential mortality between transferrin alleles in mice and voles (Ashton and Dennis 1971, Gaines *et al.* 1971, Krebs *et al.* 1973). Birdsall (1974) demonstrated an apparent excess of heterozygotes at the transferrin locus in voles.

In humans, a number of transferrin alleles have been described with apparently very few amino acid differences between them (Wang and Sutton 1965, Wang *et al.* 1966, Thymann 1978, Hoste 1979, Kühnl *et al.* 1979, Stibler *et al.* 1979). Models for transferrin variation have been complicated by the discovery of hidden alleles by iso-electric focusing. In this way the formerly most common alleles TfC, TfB, and TfD are now subdivided into families of alleles (Kühnl *et al.* 1979).

Since the discovery that the TfC allele, the most common allele in European populations (0.98), is actually three alleles with frequencies of 0.79, 0.15, and 0.04, it has become possible to test for correlations with various diseases. Beckman *et al.* (1980) have demonstrated a significant correlation between the incidence of the TfC-2 allele and spontaneous abortion.

The role of transferrins in the resistance of fish to bacterial diseases has been studied only recently. The results so far are consistent with what is known in other organisms: that there is a difference in disease resistance between allelic variants related to the physical properties of the transferrin molecule (Hershberger 1970, Suzomoto *et al.* 1977, Pratschner 1978). It is not clear how these transferrin properties interact with the other substances involved in the natural resistance of fish to disease (see Ingram 1980).

Winter *et al.* (1980) were unable to fully confirm the transferrin effects shown by Suzomoto *et al.* (1977), perhaps because of their higher experimental temperatures. More generalised relations between transferrins and general condition occur in fish like those in domesticated cattle and sheep (Balakhnin and Galagan 1972, McIntyre and Johnson 1977, Reinitz 1977).

A small number of studies have examined the biochemistry of fish transferrins (Aisen *et al.* 1972, Valenta *et al.* 1976, 1977). Valenta *et al.* (1976) found that the electrophoretic mobilities of some alleles depend on the presence of sialic acid residues on the surface of the transferrin molecule which may account for the age-dependent changes in transferrin electrophoretic patterns described by de Ligny (1968).

FISHERIES APPLICATIONS

In spite of the evidence for physiological differences between at least the most common transferrin alleles, most students of fish population genetics have assumed that the variation is neutral. The variation usually either is simply described without comment, or is assumed to have a simple stochastic distribution and is used as a statistical tool in separating stocks, or even subspecies, of fish (Møller and Naevdal 1966, Barrett and Tsuyuki 1967, Tsuyuki *et al.* 1969, Chen and Tsuyuki 1970, Utter *et al.* 1970, Wright and Atherton 1970, Payne *et al.* 1971, Wilkins 1972a, 1972b, Elson 1973, Morgan *et al.* 1973, Payne 1974, McKenzie and Martin 1975, Child *et al.* 1976, Gall *et al.* 1976, Ney and Smith 1976, Jamieson and Turner 1978, Krueger and Menzel 1979). Because the general biochemistry and physiology of transferrin variants are comparatively well known, this protein provides a good test of the validity of these applications.

The use of any allelic variants in separating stocks requires at least one assumption: the allele-frequency distribution is stationary and has a low variance. This assumption further implies that alleles do not cause differences in survival or reproduction among individuals. However, temporal variation in transferrin allele frequencies has been reported for Atlantic cod (Jamieson and Turner 1978) and

Pacific halibut, *Hippoglossus stenolepis* (Tsuyuki *et al.* 1969) (Tables 2 and 3). The range of variation of frequency in both studies is from very low to about 0.12, within the range of other loci for different year and length classes of fish (Siebenaller 1978, Smith 1979, Gauldie and Johnston 1980, Grant *et al.* 1980).

In samples of Atlantic cod (Table 2), frequencies of the common transferrin alleles TfB-2 and TfC-3 varied considerably among localities and years. How much of the total variation is time dependent?

The number of duplicate samples is too low to allow an analysis of variance, but an estimate of the contribution of the year-to-year variation to the overall variation can be made in the following way.

The overall mean frequency of the TfB-2 allele is 0.25 and the standard deviation of the distribution is 0.13 for all 13 observations. The change in TfB-2 allele frequency from year to year, measured from the first year at each station, ranged from 20% to 100% (Table 2), averaging 39 $\pm$ 27% (s.d.) for the seven observations. For a confidence interval of $\pm$ 2 s.d., the expected range of percentage changes from year to year is then 0 to 93% of the overall mean. The percentage change is measured absolutely; so the maximum expected range of the overall mean is from $0.25 - (0.93 \times 0.25)$ to $0.25 + (0.93 \times 0.25)$, at 0.018 to 0.483, whereas the minimum expected range is no change at all. The maximum expected range accommodates the highest and lowest TfB-2 frequencies, 0.48 and 0.12 respectively. By a similar approach, the expected maximum range of the TfC-3 frequency is 0.29 to 0.73.

The highest and lowest observed TfC-3 frequencies, 0.72 (Danas Bank) and 0.20 (Rittu Bank), are sufficiently far apart to suggest that the difference between them is greater than that expected from the year-to-year variation. But the other observations made at these two stations, 0.57 and 0.27, are very close to the expectation.

TABLE 2: Time-dependent variation in transferrin allele frequencies for Atlantic cod (off Atlantic Canada) (from Jamieson and Turner 1978)

			Allele frequency					
Locality	Year	$2n$*	TfA-1	TfA-2	TfB-2	TfB-3	TfB-4	TfC-1
Fyllas Bank	1966	388	0.003	0.110	0.173		0.006	0.018
	1972	224		0.103	0.219	0.004		0.004
	1973	140		0.086	0.221			0.007
Danas Bank	1972	60		0.100	0.120			
	1973	196		0.130	0.240			0.030
Nanortalik	1972	142		0.130	0.120			0.007
	1973	404		0.100	0.150		0.002	0.010
Farewell	1966	402		0.140	0.140	0.007		0.020
	1972	152		0.160	0.190			0.013
Rittu Bank	1971	210		0.005	0.360	0.009		0.210
	1975	208		0.004	0.480	0.004	0.004	0.144
Grand Bank	1967	320		0.003	0.460			0.190
	1971	198			0.370	0.010		0.100

			TfC-2	TfC-3	TfC-4	TfD-1	TfD-2	TfE
Fyllas Bank	1966	388	0.015	0.616		0.064		
	1972	224		0.592		0.076		0.004
	1973	140		0.612		0.070		
Danas Bank	1972	60		0.720		0.070		
	1973	196		0.570		0.040		
Nanortalik	1972	142		0.640		0.098		
	1973	404	0.002	0.630		0.090		
Farewell	1966	402	0.002	0.620		0.060		
	1972	152		0.570		0.070	0.007	
Rittu Bank	1971	210	0.019	0.270		0.110		
	1975	208	0.019	0.202		0.110	0.004	0.020
Grand Bank	1967	320	0.013	0.230	0.003	0.097		
	1971	198	0.040	0.320		0.120		0.040

		Percentage change in allele frequency	
		TfB-2	TfC-3
Fyllas Bank	1966–72	29	4.8
	1966–73	29	1.6
Danas Bank	1972–73	100	21
Nanortalik	1972–73	25	1.5
Farewell	1966–72	36	8
Rittu Bank	1971–75	33	26
Grand Bank	1967–71	20	39

*n = sample size; $2n$ = number of alleles sampled.

TABLE 3: Time-dependent variation in transferrin allele frequencies for Pacific halibut off Alaska (from Tsuyuki *et al.* 1969)

	Allele		
	TfA	TfB	TfC
Alitak Bay 1967	0.025	0.69	0.28
Alitak Bay 1968	0.030	0.70	0.27
Kayak I. 1967	0.025	0.72	0.25
Kayak I. 1968	0.030	0.73	0.24
Shelikoff Bay 1967	0.038	0.64	0.33
Shelikoff Bay Jul 1968	0.020	0.65	0.33
Shelikoff Bay Aug 1968	0.086	0.65	0.27
Mean allele frequency over all years and all stations	0.036	0.68	0.28

	Percentage change in allele frequency measured on first year of observation		
	TfA	TfB	TfC
Alitak Bay 1967–68	21	1.4	5.5
Kayak I. 1967–68	18	1.3	5.6
Shelikoff Bay 1967–68 Jul	47	2.0	1.0
Shelikoff Bay 1967–68 Aug	127	1.5	18.0
Mean percent year-to-year change	53.0 ± 2.51 s.d.	1.6 ± 2.30 s.d.	7.5 ± 2.73 s.d.

Expected maximum year-to-year range* around TfA mean	0.036 is 0.0–0.072
Observed maximum range from area to area around TfA mean	0.036 is 0.020–0.086
Expected maximum year-to-year range around TfB mean	0.680 is 0.665–0.690
Observed maximum range from area to area around TfB mean	0.680 is 0.640–0.730
Expected maximum year-to-year range around TfC mean	0.280 is 0.220–0.340
Observed maximum range from area to area around TfC mean	0.280 is 0.240–0.330

*Expected maximum range refers to the year-to-year variation around the mean allele frequency over all years and stations.

Transferrin variation in the Pacific halibut is not as great as that for the Atlantic cod (Table 3). Nonetheless, a crude estimate of variance based on the limited sample size shows enough time-dependent variation to accommodate the maximum differences in allele frequency for the TfA and TfC variations from different regions reported, but not for TfB variation. However, the sample sizes for the two samples with maximum difference (Kayak Island 1968 and Shelikoff Bay 1967) are such that the differences between them are not statistically significant by the Z statistic ($P < 0.10$).

All this variation is reported from samples composed of several year classes. All the samples of Atlantic cod (Jamieson and Turner 1978) and Pacific halibut (Tsuyuki *et al.* 1969) are shown to be in Hardy-Weinberg equilibrium. However, other studies have shown that aggregates of year classes may be in apparent Hardy-Weinberg equilibrium even though year classes have significant differences in allele frequency (Gauldie and Johnston 1980, Smith 1979). Indeed, Smith (pers. comm.) has shown that these individual year classes, although they are themselves in Hardy-Weinberg equilibrium, can be divided into length classes that have significant allele frequency differences, and yet these separate length classes also remain in Hardy-Weinberg equilibrium.

Additional variation due to movement of the true population boundaries probably is concealed within year classes of Atlantic cod and Pacific halibut. Thus, the variation reported by Tsuyuki *et al.* (1969) and Jamieson and Turner (1978) is the sum of the population movement variance and the year class variance, and the real variance of samples may be even greater than I have shown.

Regional differences in allele frequency still no doubt exist even with this large year-to-year variation. But detecting this location effect above the year-to-year noise is not possible without an orthogonal experimental design, which is often extremely difficult to arrange in sampling a fishery. Not only does the weather impede such rigorous sampling, but the population density and age structure vary from location to location. In addition, a sample of fish taken in a ground trawl in 15 minutes is not comparable to a sample of the same number obtained by exhaustively sampling an area for several days.

Tagging studies support my arguments against the existence of large numbers of genetically isolated stocks in both the Atlantic cod and Pacific halibut. Templeman (1979) reported tag returns from Greenland, Iceland, and Norway from cod tagged off Newfoundland. Skud (1977), after reviewing all of the Pacific halibut literature, concluded that there was convincing evidence for a single stock of Pacific halibut.

Thus, the very small differences in frequency used by Payne *et al.* (1971) and Child *et al.* (1976) to

establish celtic and boreal races for the European Atlantic salmon (0.0015 to 0.0314) must be treated with great suspicion. Without firm evidence for the absence of the year-to-year variation, it is evident that the difference between these "races", when considered in the knowledge of time-dependent variation of transferrin alleles in other fishes, is too small to accept.

MIXED-STOCK FISHERIES

The use of allele frequencies to deduce the proportions of component stocks in catches of mixed stocks presents several similar problems. The argument for assigning stock proportions depends on three variables: 1, the allele frequencies of the component stocks; 2, the allele frequency of the "mixed-stock" catch; and 3, the proportion of fish from each component stock in the mixed stock. If any two of these components are known, the third can be calculated. In practice, this usually is variable 3. This has been attempted for Columbia River chinook salmon (Milner and Teel 1979) and Alaskan sockeye salmon (Grant *et al.* 1980), both involving many genetic loci, and Atlantic salmon, based on isocitrate dehydrogenase and amino aspartate transferase variants (Cross *et al.* 1978) and transferrin variants (Fairbairn 1979, Fairbairn and Roff 1979).

The technique can be summarised in this way. Given a stock A with a frequency a of allele x, a stock B with a frequency b of allele x, and a sample C that is a mixture of both stocks with a frequency c of allele x, the proportion of stock A to stock B is given by the ratio $1 : K$ from the expression:

$$K = \frac{a}{bc}$$

The reliability of this estimate is the variance of K s_k^2. This variance is complex:

$$s_k^2 = (1/b.c)^2 \,.\, s_a^2 + (-a/c.b^2)^2 \,.\, s_b^2 + (-a/b(c)^2)^2 \,.\, s_c^2 + (1/b.c) \,.\, (-a/c(b)^2) \,.\, \mathrm{cov}\,(a,b) + (-a/c(b)^2).\,(-a/b.(c)^2) \,.\, \mathrm{cov}\,(b,c) + (1/b.c)\,(-a/b.(c)^2 \,.\, \mathrm{cov}\,(a,c).$$

The covariances (cov) are likely to be negative, but the s_k^2 will still be quite large when the year-to-year variation is taken into account. Of these stock proportion studies, only one (Cross *et al.* 1978) included an attempt to test for time-dependent variation. The difference in allele frequency at the amino aspartate transferase locus between adult Atlantic salmon and their supposed progeny (as parr) from the River Blackwater was 0.011. Single observations like this have proven to be unreliable in other studies, for example Chilcote *et al.* (1980), in which year-to-year variation proved to be quite high. This is of some concern for Blackwater salmon, for which it has already been shown that there are significant year-to-year differences in both fecundity and egg size (Pope *et al.* 1961).

Milner and Teel (1979) and Grant *et al.* (1980) presented even more unfavourable data sets. Their definition of stocks encompasses samples of widely different allele frequencies; differences within stocks were as high as 0.08 (Milner and Teel 1979) and year-to-year differences in frequency as high as 44% (Grant *et al.* 1980). The confidence limits of the estimate of component-stock proportions in Milner and Teel's study encompass 0 and 1.

Another aspect of this problem lies in the recognition that a sample contains a mixture of stocks. Fairbairn (1979) and Fairbairn and Roff (1979) rely on the Hardy-Weinberg equilibrium as an adequate test. There are good grounds to doubt the validity of this test. First, I already have noted that samples known to be mixtures can be in Hardy-Weinberg equilibrium. Second, it has been demonstrated by Lewontin and Cockerham (1959) that the chi-squared test is not very powerful in comparing observed and expected Hardy-Weinberg proportions of genotypes.

*Case Study No. 2: Allele frequencies in growth analysis**

So far, the discussion has pursued a pessimistic and negative line. This should not be interpreted to mean that allele frequency data have no place in fisheries management. The problem has been that allelic variation has been called on to ratify a hypothesis that is quite unrealistic. There is nothing in physiology, ecology, or morphology to suggest that species are divided into physically isolated, genetically homogeneous (that is "pure") strains. When we regard fishes as being "good" (in the sense of Mayr 1970) species that are broken up into local and transient genetic types depending on both migration and environmental pressure, we discover that allele data have a much more important, indeed fundamental, role to play in fisheries management that is not related to the issue of stocks. At least two management problems, growth and mortality, must be linked to allelic variation. Stock-recruit relationships provide the most formal descriptions of relative mortalities in fisheries management, but they suffer from a very high variance that increases

*This section was co-authored by W. J. Gazey of the LGL Consulting Company, Bryan, Texas.

with stock density or concentration. Thus, there is little insight to be gained by seeking correlations between allele frequencies at loci like transferrin that we know to be associated with disease—a density-dependent mortality factor—and the parameters of a stock recruitment equation. However, the variation at the high stock densities often has a Poisson distribution (variances increase with the mean) that suggests disease-related processes. In such a case, allele frequencies of those proteins involved in disease resistance may help to show whether or not some of the variance in the stock-recruit relationships has a genetic basis.

Growth of fishes provides a much more tractable relationship to allele frequencies, since data for growth curves are collected for most commercially important species.

Ricker (1973) has pointed out the problems that arise in fisheries management when physiological differences within fish populations result in differences in the growth-curve parameters that are used in productivity modelling. He showed that in such circumstances, fishing effort based on the mean growth characteristics of a mixed population will drive that population towards a monoculture of the fastest growing type. If individuals carrying different alleles grow at different rates as a consequence of physiological differences between these alleles, Ricker's (1973) multi-species problem can be extended to a multi-allele problem.

In a recent study of allelic variation in some New Zealand fishes (Gauldie and Johnston 1980), length, age, and allelic genotypes at the phosphoglucomutase (PGM) locus were recorded for a large number of tarakihi, *Cheilodactylus macropterus*, marine fish of commercial importance in the New Zealand trawl fishery. We use these data here to test the multi-allele "Ricker" effect. The samples were trawled from three stations, JO407, JO409, and JO415, off the west coast of the South Island of New Zealand. Fish were sexed and aged from otoliths read by transmitted light. Electrophoresis was carried out on cellulose acetate plates with a Helena HB barbitone buffer at pH 8.1. The PGM locus in the tarakihi has three common electrophoretically detectable alleles, fast (F), medium (M), and slow (S). Three rare alleles occur whose total frequency is much less than 1%. For this study the rare alleles have been scored as the M allele. Nonlinear estimation procedure for asymptotic length L_∞ and growth coefficient k of the von Bertalanffy curve followed the method of Gallucci and Quinn (1979). The length of fish at estimated age 0 was constrained to 0.

Gallucci and Quinn pointed out that there is always a high correlation between L_∞ and k values of the von Bertalanffy curve

$$L_t = L_\infty(1 - \exp[-k(t - t_0)])$$

irrespective of the estimation procedure. (In our study, k and L_∞ for all female tarakihi had a correlation of 0.92.) Consequently, it is necessary to evaluate the effects of differences in L_∞ before different k values can be accepted. Gallucci and Quinn devised the statistic $\omega = L_\infty \cdot k$. If differences in ω are significant, then significant differences in k must be due to effects unrelated to changes in L_∞.

For tarakihi from all three sampling stations, both ω and k differed significantly between males and females (Table 4). Such differences between the sexes also occurred within each station and, when sample sizes permitted tests, among most PGM phenotypes at each locality (Table 5).

Thus, the observed differences in the growth coefficient (k) are, with few exceptions, greater than expected by chance and are not artefacts of the correlation between k and L_∞.

The data for each of the three stations are drawn from a portion of the entire possible tarakihi growth curve, so that each population represents a different, although overlapping, part of the possible tarakihi growth curve. Because the populations sampled may never have significant numbers of individuals smaller or larger than those in the samples taken by Gauldie and Johnston (1980), the extent to which these incomplete data generate errors can be assessed only by further experiments.

TABLE 4: Parameter values associated with the von Bertalanffy growth curve for New Zealand tarakihi: L_∞ = asymptotic length (cm); k = growth constant; $\omega = k.L_\infty$

	No. in sample	Sum of squares	L_∞	k	ω
All fish	1 564	21 750.8	$39.79 \pm 2.39 \times 10^{-3}$	$0.3183 \pm 1.17 \times 10^{-6}$	$12.67 \pm 4.72 \times 10^{-5}$
Males	866	9 579.3	$38.67 \pm 3.74 \times 10^{-3}$	$0.3282 \pm 2.34 \times 10^{-6}$	$12.69 \pm 6.16 \times 10^{-3}$
Females	698	9 435.4	$42.16 \pm 8.31 \times 10^{-3}$	$0.2875 \pm 2.38 \times 10^{-6}$	$12.12 \pm 8.04 \times 10^{-3}$
t-statistic male versus female				132*	5.16*

*Significant at $P < 0.05$.

TABLE 5: Values of the von Bertalanffy growth constant k and of ω ($\omega = k.L_\infty$, where L_∞ is the asymptotic length) for tarakihi from New Zealand, according to the genotype arising from the combination of the M, S, and F alleles of phosphoglucomutase

	Males			Females			t-statistic	
							males	females
Genotypes	n	$\omega \pm$ s.d.	$k \pm$ s.d.	n	$\omega \pm$ s.d.	$k \pm$ s.d.	ω	k
Station JO407								
MM	65	13.22 ± 0.19	$0.321 \pm 0.64 \times 10^{-4}$	84	12.67 ± 0.15	$0.296 \pm 0.46 \times 10^{-4}$	7.90*	8.8*
MS	31	13.64 ± 0.45	$0.344 \pm 0.18 \times 10^{-3}$	32	12.61 ± 0.54	$0.297 \pm 0.17 \times 10^{-3}$	5.80*	NS
MF	6	12.64 ± 1.59		13	13.07 ± 5.10		0.48	
t-statistics for comparison								
MM-MS		3.14*			0.44			
Station JO415								
MM	197	12.27 ± 0.14	$0.296 \pm 0.33 \times 10^{-4}$	201	11.76 ± 0.15	$0.258 \pm 0.296 \times 10^{-4}$	12.20*	61.8*
MS	52	13.70 ± 0.32	$0.368 \pm 0.11 \times 10^{-3}$	60	11.71 ± 0.52	$0.257 \pm 0.104 \times 10^{-3}$	15.30*	58.0*
MF	27	11.41 ± 1.14	$0.228 \pm 0.23 \times 10^{-3}$	21	10.76 ± 3.09	$0.202 \pm 0.042 \times 10^{-3}$	1.35	8.8*
t-statistics for comparison								
MM-MS		142*	45.5*		0.48	0.34		
Station JO409								
MM	219	10.78 ± 0.026	$0.279 \pm 0.11 \times 10^{-3}$	107	9.83 ± 0.06	$0.229 \pm 0.16 \times 10^{-3}$	41.88*	115.9*
MS	71	11.40 ± 0.098	$0.299 \pm 0.42 \times 10^{-4}$	40	8.33 ± 0.05	$0.179 \pm 0.12 \times 10^{-3}$	59.77*	128.5*
MF	31	9.56 ± 0.132	$0.299 \pm 0.39 \times 10^{-4}$	16	11.93 ± 0.29	$0.269 \pm 0.79 \times 10^{-4}$	14.88*	15.8*
SS	8	12.80 ± 1.196	$0.363 \pm 0.70 \times 10^{-4}$	9	8.37 ± 0.35	$0.188 \pm 0.16 \times 10^{-3}$	10.71*	17.9*
t-statistics for comparison								
MM-MS		16.04*	24.54*		33.60*	75*		
MS-MF		23.90*	52.50*		24.20*	36.40*		
MM-MF		17.90*	45.20*		14.35*	16.35*		
MF-SS		8.75*	15.35*		14.60*	16.50*		
MS-SS		3.85*	7.24*		0.17	2.20*		
MM-SS		5.55*	9.45*		7.14*	9.60*		

*$P < 0.05$.

GROWTH AND PGM

Phosphoglucomutase is not a regulatory enzyme in the usual sense (that is, it is not controlled by feedback) (Stanbury *et al.* 1978). The enzyme is freely reversible with a ΔG° of about 0, and it has a high titre. The enzyme thus acts as a "hole" through which glucose-6-phosphate (G-6-P) and glucose-1-phosphate (G-1-P) can flow freely in either direction depending on the stoichiometry of the G-1-P and G-6-P concentrations. The reaction catalysed by PGM involves two substrates, G-6-P and F-2-diP (fructose-2-diphosphate), and there is a strong product inhibition effect (Ray and Roscelli 1964) that would permit modulation of the substrate competition with other enzymes that use G-6-P. Consequently, allelic variants at the PGM locus with different biochemical properties could partially reroute carbohydrates through either glycogen storage, adenosinetriphosphate production, or fatty acid synthesis, and thus influence growth rate.

MANAGEMENT IMPLICATIONS

Vooren (1973) described the population dynamics and effects of fishing pressure on the tarakihi for both the East Cape and the Kaikoura regions of New Zealand. The Pegasus Bay sample (JO409 of Gauldie and Johnston 1980) was drawn from the Kaikoura region. Before 1966 the Pegasus Bay (Kaikoura) fishery was virtually unexploited. Since 1966 the tarakihi has been heavily fished in the Pegasus Bay area and fishing pressure has been maintained in the East Cape area. Gauldie and Johnston (1980) reported allele frequencies from the 1966 to the 1973 year classes of the Pegasus Bay fishery and have unpublished frequencies from an unexploited population (Bluff) and another exploited fishery (East Cape), both for 1977. In both the Pegasus Bay series and the Bluff-East Cape comparisons, exploited populations have higher proportions of FM phenotypes and lower proportions of MS phenotype carriers (Table 6).

The von Bertalanffy growth coefficients and asymptotic lengths (in centimetres) of these two genotypes among Pegasus Bay fish are:

	Males		Females	
Genotype	k	L_∞	k	L_∞
FM	0.226	42	0.268	45
MS	0.299	38	0.179	46

Tong and Vooren (1972) report that among tarakihi an excess of the small fish which are caught are males, and the largest fish are virtually all females (Fig. 3). Male MS individuals are the fastest growing, so that the catch data indicate that MS males are likely to be depleted in number as a result of fishing pressure. In addition, the "fishing down" effect—the rapid removal of the largest available fish—will be at the expense of the large, slow-growing females, the MS females. As a result, FM phenotypes with intermediate growth will predominate if a population is heavily fished.

Thus, one interpretation of the decrease in MS genotypes and the increase in FM genotypes is that a multi-allele "Ricker" effect has taken place in the

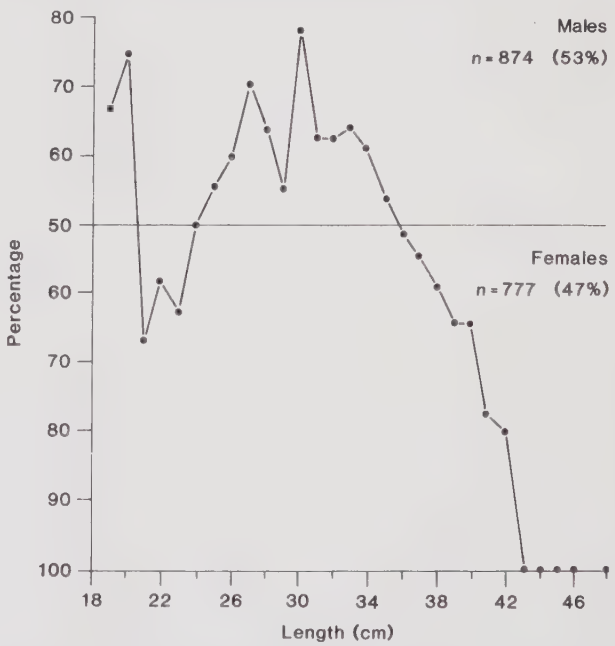

Fig. 3: Sex ratio changes of tarakihi reported by Tong and Vooren (1972), over different length classes. The numbers and percentages of males and females refer to the total number in the entire sample.

TABLE 6: Frequencies of phosphoglucomutase phenotypes (combinations of F, M, and S alleles) in unexploited and exploited populations of New Zealand tarakihi (from Gauldie and Johnston 1980 and unpublished data)

	Phenotype					
Population	FF	FM	MM	MS	SS	FS
Unexploited Pegasus Bay, 1966	0.03	0.07	0.62	0.24	0.03	0.00
Exploited Pegasus Bay, 1973	0.00	0.20	0.60	0.18	0.03	0.00
Unexploited Bluff, 1977	0.02	0.05	0.62	0.27	0.04	0.00
Exploited East Cape, 1977	0.02	0.14	0.64	0.17	0.01	0.01

Pegasus Bay tarakihi population as a result of inherited differences in growth rate associated with allele genotypes at the PGM locus.

However, there are other possible explanations. Gauldie and Johnston (1980) suggest that there may be a general north-south cline in PGM allele frequency for tarakihi correlated with temperature. Temperature differences of about 3.5 °C occur between East Cape and Bluff. Similar temperature differences at the time of spawning in 1966 and 1973 may have caused a difference in allele frequency of those year classes similar to that described by Smith (1979) for the esterase locus in the snapper *Chrysophrys auratus*.

The relationship between growth, allelic phenotype, and fishing pressure may not be so readily demonstrated for other enzymes and other species. But it is reasonable to expect that electrophoretic studies that pursue the identification of stocks from small samples, and that assume that alleles are neutral markers of the genome, run the risk of losing valuable information, information that is immediately relevant to the conservation and wise utilisation of fisheries.

CONCLUSION

Population geneticists, in addressing the question of stocks of fish, have generally concerned themselves only with the statistical properties of population allele frequencies. Stocks, defined as genetically isolated populations, have thus been measured in terms of assumed statistical models. The evidence presented here indicates the extent of the failure of the biological properties of alleles to map on to the mathematical properties of the simple statistical models used in stock identification. This failure has serious implications in fisheries management modelling. The simple stock identification models have much in common with other statistical models used in fisheries management. Fundamentally, all fisheries management models assume that the population is homogeneous with respect to traits like growth, recruitment, and mortality. Variations in these traits are regarded as being scholastic, introduced by changes in external factors that are more or less random. It is clear that this assumption is incorrect. Populations of fish must be regarded as mixtures of types that have different genetic programmes for at least traits like growth and disease resistance, in which case some of the observed variation must be due to the fundamental heterogeneity of populations with respect to those traits. Such mixtures are not compatible with any sort of parametric statistics, since parametric statistics require that populations have single, true mean value, or the relationship between the mean values of components be linear and additive. Worse still, these mixtures are likely to change under fishing pressure.

Fisheries managers can no longer model fisheries with only the classical quantitative fisheries management considerations—recruitment numbers, growth rates, mortality rates, total allowable catch—in mind. Attention must be paid to the qualitative behaviour of fisheries resources that can undergo biochemical and physiological changes in the face of natural and man-imposed stress. Modelling techniques are already available (Walker *et al.* 1981, Ludwig *et al.* 1978) and allelic variation can provide many direct experimental data to sustain such models.

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